Isolated Perfused Rabbit Lung As a Model for Intravascular and Intrabronchial Administration of Bronchodilator Drugs I: Isoproterenol

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Abstract □ The absorption, uptake, and metabolism of isoproterenol was studied following intravascular, intrabronchial, and aerosol administration of the drug to the isolated perfused rabbit lung. Capacity-limited metabolism of isoproterenol was observed following the addition of five doses, ranging from 10^{-7} to 10^{-5} moles, directly into the circulation of the lung system. A physiologically based perfusion model was developed to describe the disposition of the drug and metabolite in the isolated lung preparation. This model was also used to analyze data collected following intrabronchial and aerosol administration of isoproterenol.

Keyphrases □ Isoproterenol—intravascular and intrabronchial administration, isolated perfused rabbit lung □ Bronchodilators—isoproterenol, intravascular and intrabronchial administration, isolated perfused rabbit lung □ Pharmacokinetics—isolated perfused rabbit lung as a model for intravascular and intrabronchial administration of isoproterenol

Isoproterenol is often administered directly into the lungs by aerosol inhalation in the treatment of bronchial asthma. Little of the inhaled dose actually reaches the airways (1), but that which does is responsible for the bronchodilating effect. Little is known of the fate of the small fraction which reaches the airways and of the role of the lung in the disposition of the drug. The objective of this study was to evaluate the absorption, uptake, and metabolism of isoproterenol in the isolated perfused rabbit lung following intravascular, intrabronchial, and aerosol administration.

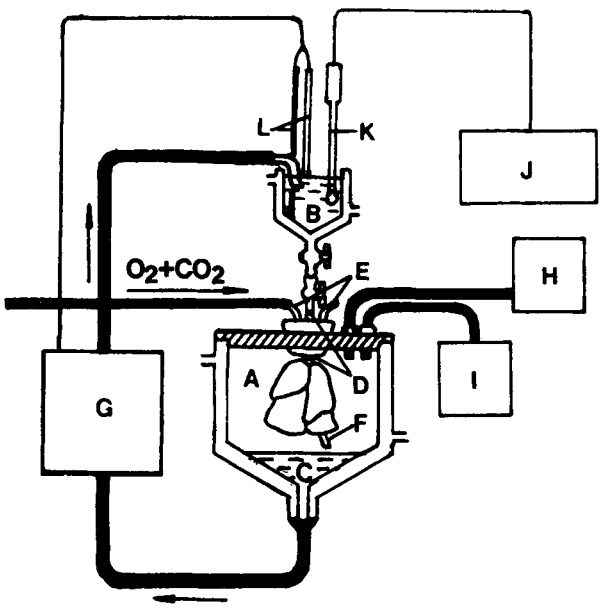

Figure 1—*Diagram of the isolated perfused rabbit lung apparatus. Key: (A) lung chamber; (B) upper reservoir; (C) lower reservoir; (D) pulmonary artery cannula; (E) tracheal cannula; (F) pulmonary venous cannula; (G) perfusion pump; (H) animal respirator; (I) pressure gauges; (J) pH meter; (K) pH electrode; (L) level-sensing probes.*

The isolated perfused rabbit lung preparation offers an ideal experimental system for studying the disposition of drugs by the lung, as it allows the lung to be studied independent of the rest of the body. The design of the system facilitates rapid and convenient sample withdrawal and complete recovery of the lung tissue and perfusate. Viability of the isolated perfused rabbit lung preparation has been documented by physiological, hematological, histological, and biochemical methods for periods up to 4 hr (2–5). Similar preparations have been used in the past to study the uptake and metabolism of various pharmacological agents. Little has been done, however, to apply pharmacokinetic concepts to the analysis of the data generated from these studies.

Previous experiments dealing with the disposition of isoproterenol in isolated lung preparations indicate that the drug is not taken up extensively by the lung (6) and that 3-*O*-methylisoproterenol is the only metabolite formed by the lung (7). The drug appears to be well-ab-

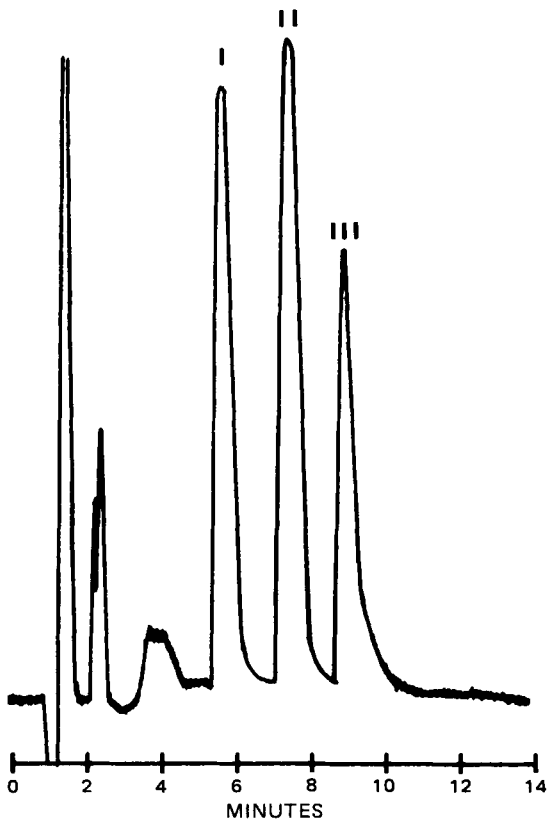

Figure 2—*Sample chromatogram of a diethylhexylphosphoric acid perfusate extract containing (I) isoproterenol, (II) isoproterenone, and (III) 3-O-methylisoproterenol separated on a strong cation exchange column with a phosphate buffer–methanol mobile phase.*

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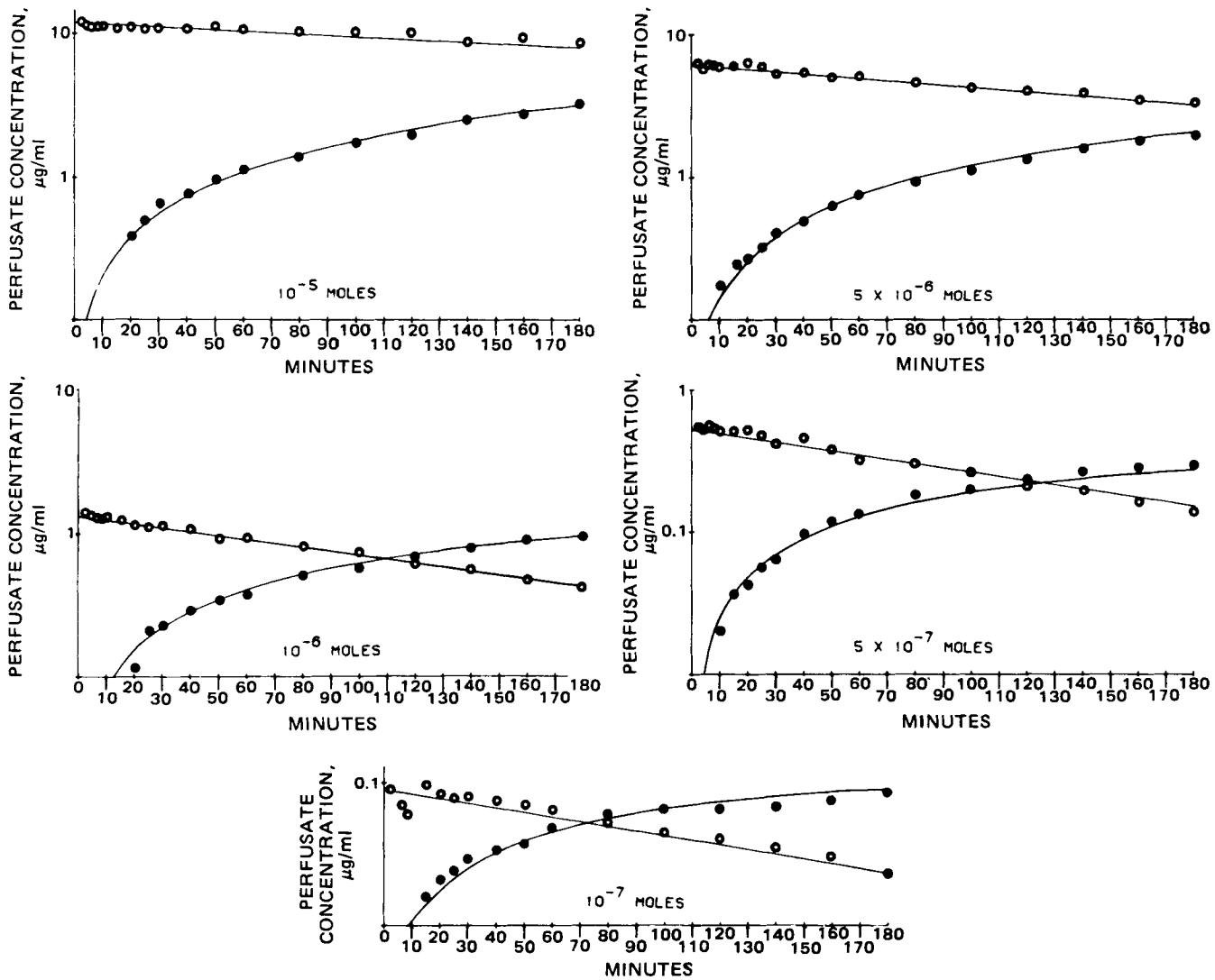

Figure 3—*Semilogarithmic plots of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol, comparing observed values with those predicted by compartmental model fits for five intravascular doses of isoproterenol hydrochloride administered to the isolated perfused rabbit lung.*

sorbed when administered directly into the airways, and a 30% first-pass *O*-methylation during absorption has been reported (7).

EXPERIMENTAL

Isolated Perfused Rabbit Lung—The isolated perfused rabbit lung system has been described previously (2, 3). A schematic drawing of the apparatus is shown in Fig. 1. Male New Zealand rabbits (3–5 kg) were anesthetized with sodium pentobarbital and treated with heparin. The animals were exsanguinated *via* cardiac puncture; the heart, lungs, and trachea were removed; and the trachea, pulmonary artery, and left atrium were cannulated. The lung preparation was suspended in a cylindrical thorax[1] and connected to an upper reservoir such that perfusion medium could flow from the reservoir through the pulmonary vasculature and exit *via* a cannula inserted in the left atrium. The pooling perfusate was pumped back to the reservoir by a perfusion pump[2], providing constant circulation through the lung system. The lungs were ventilated with warm humidified air containing carbon dioxide by operating a small animal respirator[2] to create an alternating negative pressure within the lung chamber. This resulted in expansion and contraction of the lung and inspiration and expiration much like that in the intact animal. The thorax and the reservoir were water-jacketed to regulate the temperature at 37°. Once the system was in operation, it was allowed to equilibrate 15–20 min prior to drug administration. Sampling (1-ml specimens) was from the upper reservoir. For each perfusion, Krebs-Ringer bicarbonate solution (150 ml) containing 4.5% bovine serum albumin and the following in grams per liter was used: NaCl, 6.57; KCl, 0.355; $CaCl_2$, 0.28; KH_2PO_4, 0.16; $MgSO_4$, 0.145; $NaHCO_3$, 2.1; and glucose, 0.18. The pH was adjusted to 7.4 with sodium hydroxide.

Drug Administration—Isoproterenol hydrochloride was administered to the isolated lung system by intravascular, intrabronchial, and aerosol administration. One milliliter aliquots of aqueous isoproterenol hydrochloride solutions containing 10^{-5}, 5×10^{-6}, 10^{-6}, 5×10^{-7}, and 10^{-7} moles/ml were added to the upper reservoir for intravenous administration. For intrabronchial dosing, 250-μl aliquots containing 10^{-5} and 10^{-6} moles of drug were delivered to the airways as a bolus *via* a small bore plastic tubing inserted through the tracheal cannula. For aerosol administration, a 50-mg/ml aqueous solution of isoproterenol hydrochloride was nebulized[3] and delivered to the airways through a plastic tubing attached to the tracheal cannula for a 1-min dosing period. During drug administration, the respirator controls were changed from the normal settings of 30-ml stroke volume and 50% inspiration to 50-ml stroke volume and 70% inspiration. This maximized the flow of aerosolized drug into the lung by causing deep inspirations.

Chromatography—The perfusate concentrations of isoproterenol and 3-*O*-methylisoproterenol[4] were determined by high-pressure liquid chromatography (HPLC). The compounds were separated on a strong cation exchange column[5] and detected with a variable wavelength UV

[1] Fabricated by Thomas Burcar, Cincinnati, Ohio.
[2] Harvard Apparatus, So. Natuck, Mass.
[3] Model 35B Ultrasonic Nebulizer, DeVilbiss Co., Somerset, Pa.
[4] Provided by C. H. Boehringer Sohn, Postfach 200, Germany.
[5] PXS 10/25 SCX, Whatman, Inc., Clifton, N.J.

Table I—Parameter Estimates Generated by a Composite Fit of the Intravascular Isoproterenol Data to the Compartmental Model

Dose, moles	Vm μg/ml min	Km, μg/ml	V_1, ml	V_2, ml	SS[a] Isoproterenol	r^2 Isoproterenol	SS 3-O-Methylisoproterenol	r^2 3-O-Methylisoproterenol
10^{-5}	0.021	1.95	206	199	146.0	0.997	0.084	0.997
5×10^{-6}	0.021	1.95	200	262	39.6	0.992	0.025	0.996
10^{-6}	0.021	1.95	177	198	1.98	0.995	0.012	0.992
5×10^{-7}	0.021	1.95	209	345	0.362	0.984	0.0011	0.985
10^{-7}	0.021	1.95	250	247	0.013	0.387	0.0002	0.943

[a] Sum of squared deviations.

detector[6]. Due to the column-to-column variability and the tendency of the columns to lose efficiency during use, various mobile phases were utilized during the study. Potassium phosphate buffers (0.003–0.03 M) containing 5–30% methanol (pH 3.0) were used at a flow rate of 2 ml/min. The compounds were detected at either 278 or 220 nm. The higher wavelength was used when large concentrations of isoproterenol were present and isoproterenone hydrochloride[7] was used as an internal standard. A sample chromatogram is shown in Fig. 2. Detection at 220 nm afforded greater sensitivity, but the chromatographic conditions needed to separate isoproterenol and 3-O-methylisoproterenol from interfering extraneous peaks precluded the use of isoproterenone as the internal standard; morphine hydrochloride was used as the standard in this case.

Extraction of Perfusate Samples—All procedures were performed under yellow light to protect isoproterenol against photodecomposition. To each sample was added 1 ml of 0.1 N acetate buffer (pH 4.0) containing internal standard (isoproterenone hydrochloride or morphine hydrochloride) and 3 ml of 5% (v/v) diethylhexylphosphoric acid in freshly distilled diethyl ether. This was vortexed 1 min and centrifuged, and the ethereal layer was decanted into a conical centrifuge tube. To this was added 25–100 μl of 1 N HCl with 1% ascorbic acid, and the mixture was vortexed 1 min and centrifuged. An aliquot of the aqueous phase was injected into the chromatographic system.

Standard curves were prepared for each experiment by spiking 1-ml blank perfusate samples with 100 μl of 1% ascorbic acid and varying concentrations of isoproterenol and 3-O-methylisoproterenol. These standards were extracted and analyzed in the same manner as experimental samples.

Preparation of Lung Homogenate Samples—The lungs were thawed and blotted dry. Extraneous tissue was removed and the lung was weighed. The tissue was cut into small slices, diluted with 40 or 60 ml of normal saline containing 1% ascorbic acid, and homogenized[8]. A 1-ml aliquot of the homogenate was then extracted in the same manner as the perfusate samples previously described. Standard curves were prepared by adding varying amounts of isoproterenol and 3-O-methylisoproterenol to blank homogenate samples and analyzing these along with the unknown samples.

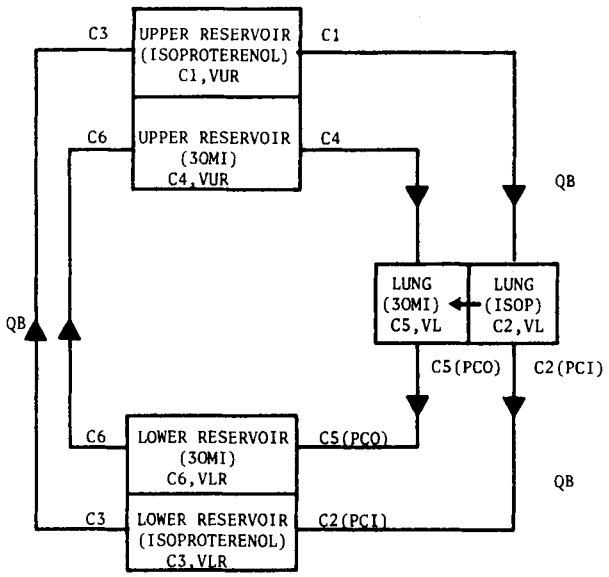

Figure 4—*Perfusion model used to describe the disposition of isoproterenol and 3-O-methylisoproterenol by the isolated perfused rabbit lung.*

[6] Vari-Chrom, Varian Associates, Palo Alto, Calif.
[7] Synthesized in this laboratory.
[8] Model SDT-182 Tissumizer, Tekmar Co., Cincinnati, Oh.

RESULTS

Intravascular Administration—Five doses of isoproterenol hydrochloride ranging from 10^{-7} to 10^{-5} moles were administered directly into the circulating perfusion medium, and samples were withdrawn for 180 min and analyzed for isoproterenol and 3-O-methylisoproterenol. In each experiment, isoproterenol concentrations appeared to decline monoexponentially with a corresponding increase in metabolite levels. The apparent half-life for drug elimination from the perfusate decreased with decreasing dose as the fraction of the dose appearing as 3-O-methylisoproterenol increased. This suggested a capacity-limited process for metabolism of isoproterenol. To investigate this possibility, data were analyzed pharmacokinetically using both a compartmental and perfusion model.

Compartmental Analysis—Each set of isoproterenol and 3-O-methylisoproterenol perfusate concentration–time data were fitted to a simple one-compartment model with the metabolism of isoproterenol to 3-O-methylisoproterenol being described by the Michaelis–Menten equation. The rates of change of drug and metabolic concentrations were described by the differential equations:

$$\frac{dC_1}{dt} = -\frac{Vm \times C_1}{Km + C_1} \quad \text{(Eq. 1)}$$

$$\frac{dC_2}{dt} = \frac{(Vm \times C_1)V_1}{(Km + C_1)V_2} \quad \text{(Eq. 2)}$$

where

C_1 = perfusate concentration of isoproterenol (μg/ml);
C_2 = perfusate concentration of 3-O-methylisoproterenol (μg/ml);
Vm = theoretical maximum metabolic rate (μg $ml^{-1}min^{-1}$);
Km = Michaelis constant (μg/ml);
V_1 = apparent volume of distribution of isoproterenol (ml);
V_2 = apparent volume of distribution of 3-O-methylisoproterenol (ml).

The isoproterenol and 3-O-methylisoproterenol perfusate concentration–time data from the intravascular studies were fitted to the differential equations using the NONLIN (8) digital computer program for nonlinear least-squares regression. The entire set of data (*i.e.*, isoproterenol and 3-O-methylisoproterenol from all five experiments) were fitted to this model resulting in unique estimates of Vm and Km describing the metabolism over the entire range of doses, whereas, V_1 and V_2 were allowed to vary for each dose independent of the others. Table I shows the parameter estimates obtained from these fits. Semilogarithmic plots comparing the observed data to that predicted from the composite fit are shown in Fig. 3.

Perfusion Model—A perfusion model was developed to describe the isolated perfused rabbit lung system and to assist in the analysis and interpretation of the observed data.

Figure 4 depicts the isolated perfused lung model as used to describe the concentration–time course for isoproterenol and 3-O-methylisoproterenol following intravascular administration of isoproterenol. This model is based on the following assumptions: (*a*) the concentration of isoproterenol and 3-O-methylisoproterenol entering the lung vasculature is the same as that in the upper reservoir; (*b*) the concentration of the two compounds in the lung tissue is in equilibrium with that in the perfusion medium leaving the lung and entering the lower reservoir; (*c*) the concentration entering the upper reservoir is the same as that leaving the lower reservoir; (*d*) the metabolism of isoproterenol to 3-O-methylisoproterenol occurs in the lung tissue by a capacity-limited process described by the Michaelis–Menten equation.

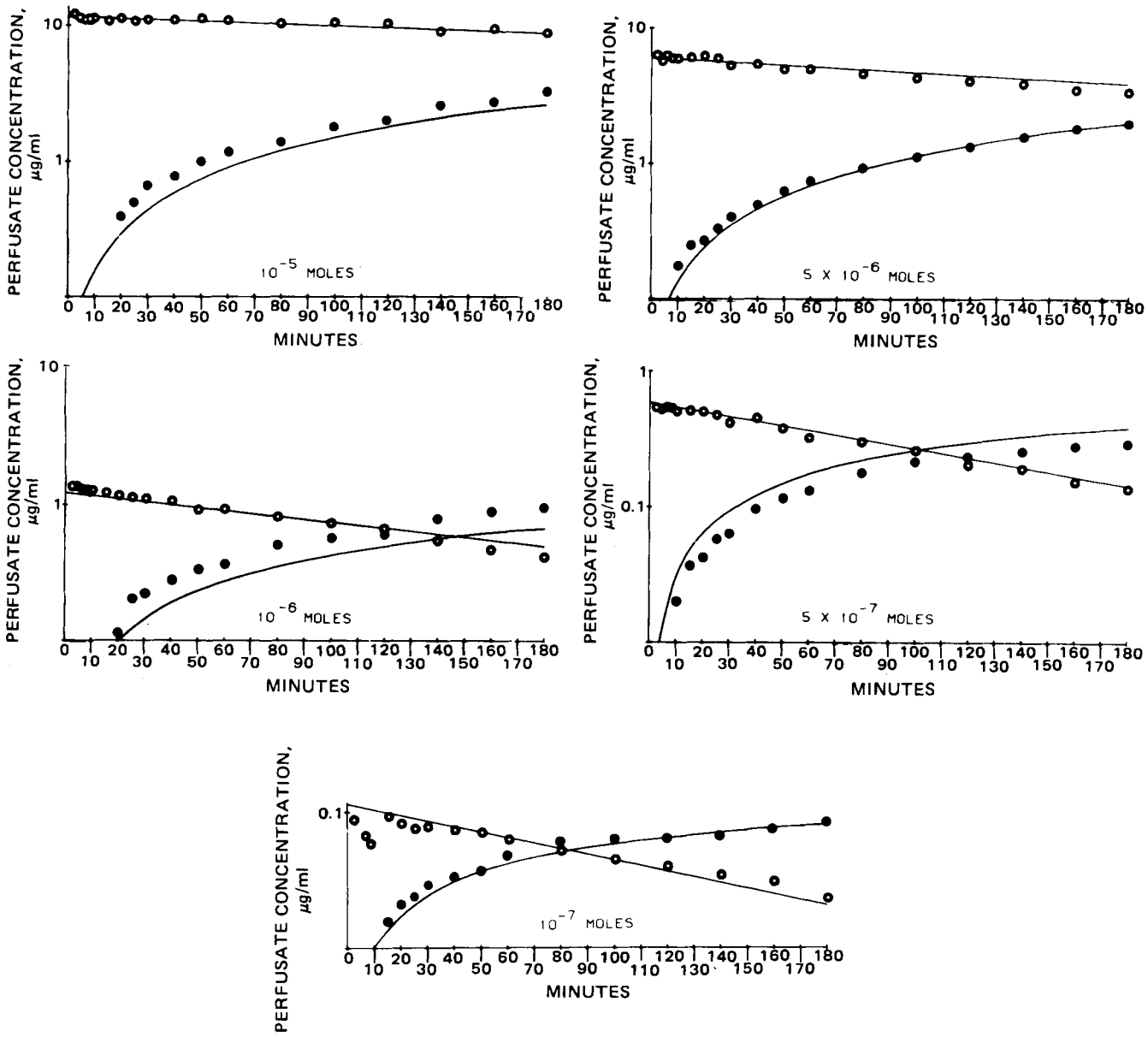

Figure 5—*Semilogarithmic plots of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol comparing observed values with those predicted from perfusion model simulations for five intravascular doses of isoproterenol administered to the isolated perfused rabbit lung.*

The following differential equations describe the rate of change of isoproterenol and 3-*O*-methylisoproterenol concentrations in the lung tissue and in the reservoir compartments following introduction of a bolus of isoproterenol into the upper reservoir:

$$\frac{dC_1}{dt} = \frac{Q_b}{V_{UR}}(C_3 - C_1) \qquad \text{(Eq. 3)}$$

$$\frac{dC_2}{dt} = \frac{Q_b \times C_1}{V_L} - \frac{Q_b \times C_2}{PC_I \times V_L} - \frac{Vm \times C_2}{Km + C_2} \qquad \text{(Eq. 4)}$$

$$\frac{dC_3}{dt} = \frac{Q_b \times C_2}{PC_I \times V_{LR}} - \frac{Q_b \times C_3}{V_{LR}} \qquad \text{(Eq. 5)}$$

$$\frac{dC_4}{dt} = \frac{Q_b(C_6 - C_4)}{V_{UR}} \qquad \text{(Eq. 6)}$$

$$\frac{dC_5}{dt} = \frac{Q_b \times C_4}{V_L} - \frac{Q_b \times C_5}{PC_0 \times V_1} + \frac{Vm \times C_2}{Km + C_2} \qquad \text{(Eq. 7)}$$

$$\frac{dC_6}{dt} = \frac{Q_b \times C_5}{PC_0 \times V_{LR}} - \frac{Q_b \times C_6}{V_{LR}} \qquad \text{(Eq. 8)}$$

where

C_1 = concentration of isoproterenol in the upper reservoir (μmoles/ml);
C_2 = concentration of isoproterenol in the lung tissue (μmoles/ml);
C_3 = concentration of isoproterenol in the lower reservoir (μmoles/ml);
C_4 = concentration of 3-*O*-methylisoproterenol in the upper reservoir (μmoles/ml);
C_5 = concentration of 3-*O*-methylisoproterenol in the lung (μmoles/ml);
C_6 = concentration of 3-*O*-methylisoproterenol in the lower reservoir (μmoles/ml);
V_{UR} = volume of the upper reservoir (ml);
V_L = volume of the lung (ml);
V_{LR} = volume of the lower reservoir (ml);
PC_I = apparent partition coefficient for isoproterenol between the lung tissue and the emergent perfusion medium;
PC_0 = apparent partition coefficient for 3-*O*-methylisoproterenol between the lung tissue and the emergent perfusate;
Vm = maximum rate of metabolism of isoproterenol to 3-*O*-methylisoproterenol in the lung tissue (μmoles $ml^{-1}min^{-1}$);
Km = Michaelis constant (μmoles/ml);
Q_b = rate of flow of perfusion medium (ml/min).

The parameters used to describe this model were derived from actual

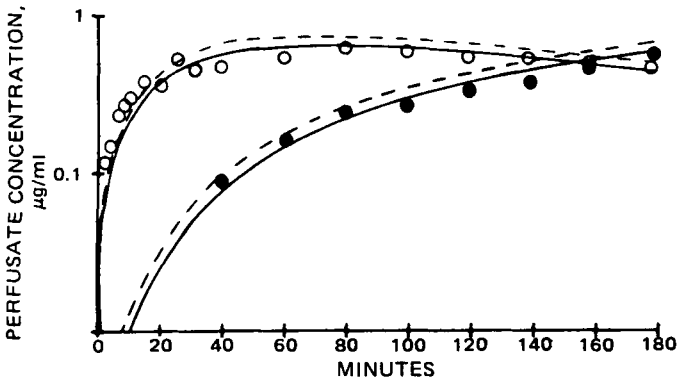

Figure 6—*Semilogarithimic plot of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol following intrabronchial administration of 10^{-6} moles of isoproterenol assuming (- - -) 100% and (——) 90% absorption.*

experimental conditions associated with the isolated lung system. The values for V_{UR}, V_{LR}, PC_I, PC_0, Vm, Km, and Q_b were the same for all simulations. Only the dose and V_L were varied. The volumes of the upper and lower reservoirs were measured as 62 and 88 ml, respectively. The flow rate was constant at 150 ml/min. The partition coefficients for isoproterenol and 3-*O*-methylisoproterenol were determined experimentally from several experiments and an average value for each was used (1.5 for isoproterenol and 3.8 for 3-*O*-methylisoproterenol). Michaelis–Menten constants were estimated from those obtained with the compartmental model fits and were adjusted to represent metabolism occurring from the lung tissue. The following conversions were used to determine these constants from those reported in Table I:

$$Vm^* = \frac{Vm \times V_1}{MW \times V_L} \qquad \text{(Eq. 9)}$$

$$Km^* = \frac{Km \times PC_I}{MW} \qquad \text{(Eq. 10)}$$

where

Vm and Km = Michaelis–Menten constant generated from the composite compartmental fit assuming metabolism from the central compartment (perfusate);
Vm^* and Km^* = Michaelis–Menten constants representing metabolism from the lung tissue;
V_1 = volume of the central compartment of the compartmental model;
V_L = volume of the lung;
MW = molecular weight of isoproterenol;
PC_I = apparent partition coefficient for isoproterenol.

The Vm obtained from this conversion was 0.001 µmoles $ml^{-1}min^{-1}$ while the Km was 0.014 µmoles/ml. The weight of each lung was used as the lung volume.

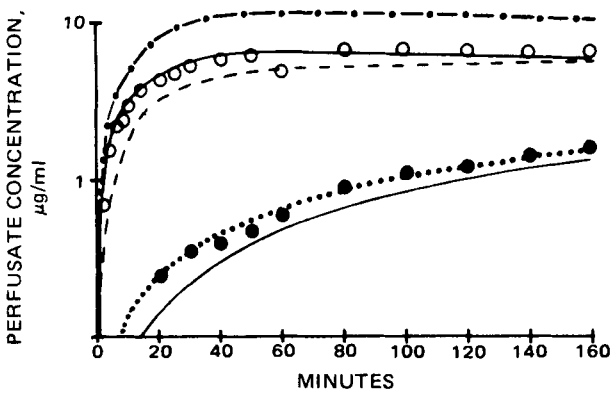

Figure 7—*Semilogarithmic plot of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol following intrabronchial administration of 10^{-5} moles of isoproterenol hydrochloride. Perfusion model predictions for isoproterenol assuming (- · · · · ·) 100% and (——) 60% absorption are shown along with predicted 3-O-methylisoproterenol profiles assuming (- - - -) 40%, (·····) 2.5%, and (——) no first-pass metabolism.*

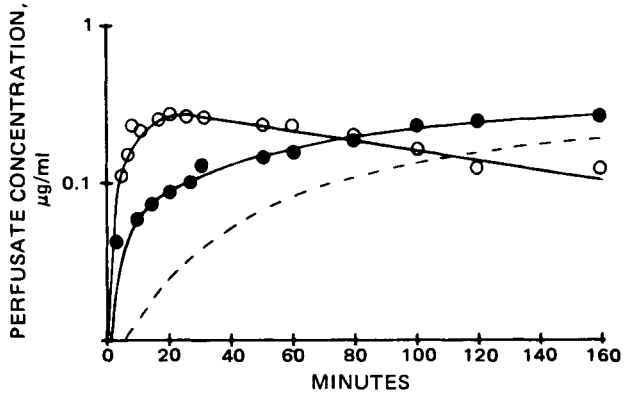

Figure 8—*Semilogarithmic plot of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol following aerosol administration of isoproterenol hydrochloride. Perfusion model predictions for the absorption of 2.75×10^{-7} moles of (——) isoproterenol are shown along with predicted 3-O-methylisoproterenol concentrations with (——) and without (- - - -) 20% first-pass metabolism.*

These equations and parameter estimates were used to produce simulations of the isoproterenol and 3-*O*-methylisoproterenol concentration–time profile in the upper reservoir of the isolated lung system following administration of isoproterenol into the upper reservoir. These simulations were compared to the observed data to determine how the model actually described that which was occurring. These comparisons are shown in Fig. 5. There was good agreement for the isoproterenol data, whereas, slight deviations from the observed metabolite data are observed in some cases.

Intrabronchial Studies—The absorption and subsequent metabolism of isoproterenol was studied following endotracheal instillation of aqueous solutions containing 10^{-5} and 10^{-6} moles of isoproterenol hydrochloride. In both cases the absorption was relatively slow with peak drug levels occurring at 60–80 min. Comparison of isoproterenol and 3-*O*-methylisoproterenol concentrations to those observed following equivalent intravascular doses suggest that less drug and metabolite appeared in the circulation following intrabronchial administration. Determining the fraction of the dose absorbed by the usual method of comparing areas under the curves was not possible, because an accurate estimate of the area under the curve could not be obtained due to nonlinear pharmacokinetics.

The perfusion model was used to assist in the interpretation of the data. The only modification from the previous model was the addition of a first-order pathway for the absorption of isoproterenol from the airways into the lung tissue. In this case the dose was entered into the absorption site rather than the upper reservoir. All other parameters and estimates were identical to those used in the previous flow model.

The simulations produced by this model adequately matched the 10^{-6} mole dose (Fig. 6) but predicted higher isoproterenol concentrations than actually observed for the 10^{-5} mole dose (Fig. 7). The intravascular data suggested that if first-pass metabolism were occurring, it would most likely be due to conversion of isoproterenol to 3-*O*-methylisoproterenol as the drug was being absorbed. This preabsorptive first-pass metabolism was incorporated into the model as the absorption of a fraction of the dose as the metabolite (with the same rate of absorption as isoproterenol). Simulations involving various fractions of the dose being absorbed as intact isoproterenol and as 3-*O*-methylisoproterenol were compared to the observed data to determine if the first-pass model could describe the data.

The results of some of these simulations for the 10^{-5} mole dose are shown in Fig. 6. An absorption rate constant of 0.056 min^{-1} was used in these simulations. Comparing the simulations to the observed data shows that the model with 60% of the dose being absorbed intact and 2.5% as the metabolite best agrees with the data. This suggests that the intrabronchial dose was not totally absorbed but does not indicate an extensive first-pass effect. Similar simulations were produced for the 10^{-6} mole dose. The best agreement with the observed data was achieved with a model assuming that 90% of the dose was absorbed as isoproterenol with no first-pass effect occurring (Fig. 6). An absorption rate constant of 0.025 min^{-1} was used in these simulations.

Aerosol Studies—Isoproterenol hydrochloride was administered to the lung as a nebulized aqueous solution and the resulting concentrations of drug and metabolite were determined. Absorption following aerosol administration was faster than that with intrabronchial administration

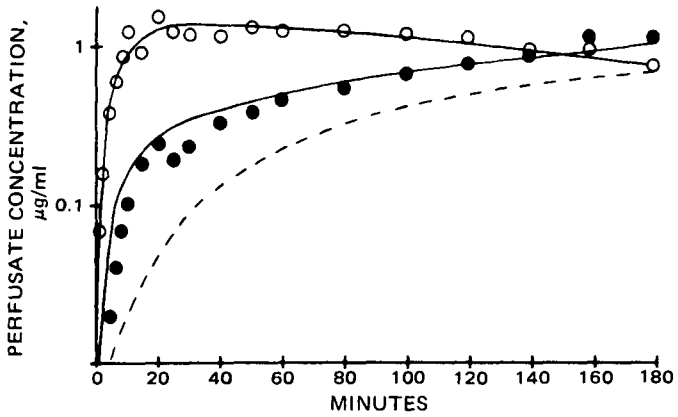

Figure 9—*Semilogarithmic plot of concentration* versus *time for (○) isoproterenol and (●) 3-O-methylisoproterenol following aerosol administration of isoproterenol hydrochloride. Perfusion model predictions for the absorption of 1.3 × 10^{-6} moles of (—) isoproterenol are shown along with predicted 3-O-methylisoproterenol concentrations with (—) and without (- - -) 16% first-pass metabolism.*

(Figs. 8 and 9). The *O*-methyl metabolite also appeared at a faster rate than that following intrabronchial administration. Data were analyzed in the same manner as were the intrabronchial data. The dose delivered to the lung by the nebulizer was not known and was estimated by the simulations. Simulations representing absorption of various fractions of the dose as the metabolite were generated and compared to the observed data. Figure 8 shows observed isoproterenol and 3-*O*-methylisoproterenol concentrations compared to that predicted for the absorption of 2.75 × 10^{-7} moles of aerosolized isoproterenol with and without a simulated 20% first-pass effect represented as absorption of the metabolite. An absorption rate constant of 0.12 min^{-1} was employed for both drug and metabolite. In Fig. 9, the simulated amount of drug absorbed was 1.3 × 10^{-6} moles while that of 3-*O*-methylisoproterenol was 2.5 × 10^{-7} moles (16% of the dose). The absorption rate constant for both was 0.08 min^{-1}. In both cases the incorporation of the first-pass phenomenon into the model provided simulations which matched the observed data much better than that seen when the phenomenon was not included in the model. Although this in itself is not sufficient documentation for concluding that first-pass metabolism was occurring, it does support similar findings by other investigators (7).

Lung Uptake—Lungs were homogenized and analyzed for isoproterenol and 3-*O*-methylisoproterenol. Partition coefficients were calculated for isoproterenol and the metabolite by dividing the lung concentration by the concentration in the final perfusate sample. The mean partition coefficient for isoproterenol was 1.49 ± 0.56 (n = 5), while that for the *O*-methyl metabolite was 3.79 ± 0.66 (n = 7). A plot of the amount of drug in the lung as a function of perfusate concentration for the two (Figs. 10 and 11) shows that uptake was linear over a 100-fold range of concentrations for both drug and metabolite.

DISCUSSION

The results of these studies suggest that pulmonary disposition is of little significance in the overall systemic clearance of isoproterenol when the drug is administered orally or parenterally. When administered *via* the airways, however, isoproterenol may be subject to first-pass metabolism, and the lung may play a significant role in the overall disposition of the drug.

Isoproterenol was metabolized in the lung tissue to 3-*O*-methylisoproterenol. The rate and extent of metabolism was dependent on the amount of drug administered to the lung system, and the kinetics of this process could be described by the Michaelis–Menten equation. The pulmonary clearance of the drug following intravascular administration of the lowest dose (10^{-7} moles) was ~2 ml/min. Lower doses would not be expected to be cleared much faster. Accumulation of the drug by the lung was limited, with concentrations in the lung tissue ~1.5 times those in the perfusion medium. The perfusate concentration profiles following intravascular administration displayed no discernible distribution phase, suggesting rapid equilibration of drug between the perfusate and lung tissue.

Interpretation of the data following intrabronchial and aerosol administration was complicated by the capacity-limited metabolism of isoproterenol. Reports in the literature have suggested a 30% first-pass

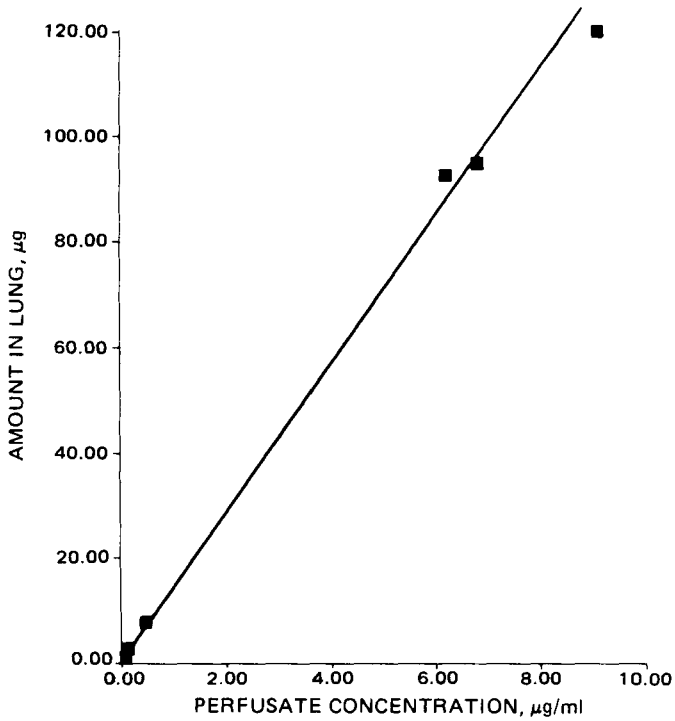

Figure 10—*Uptake of isoproterenol by the lung.*

metabolism of isoproterenol to its *O*-methyl metabolite following administration of the drug *via* the airways (7). Usual methods for evaluating first-pass metabolism (*i.e.*, *AUC* comparisons) were not applicable, as accurate estimates of infinity *AUC* values could not be obtained. As an alternative for interpreting the data, a mathematical model similar to the perfusion model described previously (9) was developed, based upon mass balance and the flow of perfusion medium through the various compartments of the isolated perfused lung system. The model described the drug and metabolite data following intravascular administration of isoproterenol.

Before the perfusion model could be modified to simulate first-pass metabolism, the nature of the first-pass effect had to be established. Two possible mechanisms for first-pass metabolism were discussed previously in a review of presystemic drug elimination (10). Postabsorptive first-pass metabolism occurs when a drug is absorbed into an eliminating organ; the fraction eliminated presystemically is equivalent to the organ extraction ratio (Cl/Q_h) following systemic administration (where Cl is the

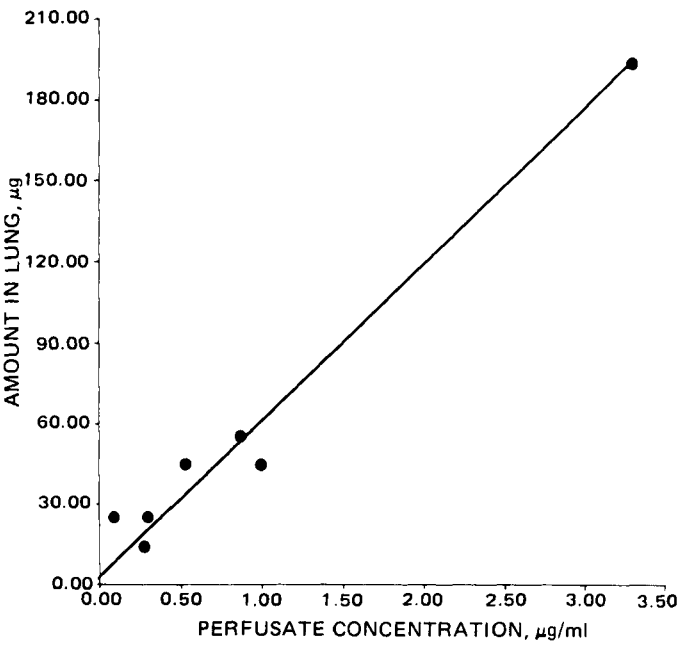

Figure 11—*Uptake of 3-O-methylisoproterenol by the lung.*

organ clearance of the drug following systemic administration, and Q_b is the flow rate through the organ). In this case the fraction of the absorbed dose reaching the circulation intact (F) is given as:

$$F = 1 - Cl/Q_b \qquad \text{(Eq. 11)}$$

Preabsorptive first-pass metabolism involves metabolism of a drug as it is absorbed into the circulation from the site of administration. In this case the fraction eliminated presystemically is not equal to the systemic extraction ratio.

The maximum pulmonary clearance of isoproterenol following systemic administration to the rabbit lung was ~2 ml/min, representing an extraction ratio of ~0.01. If only postabsorptive first-pass metabolism were occurring, this would predict that 99% of an intrabronchial or aerosol dose of isoproterenol should reach the circulation intact. If >1% first-pass metabolism occurs, as has been reported (7), preabsorptive first-pass metabolism must be involved. The preabsorptive metabolism would be observed experimentally as absorption of the metabolite into the circulation along with the parent drug. This was incorporated into the perfusion model as first-order absorption of both drug and metabolite, and various simulations with this model were compared to the experimental results following intrabronchial and aerosol administration of isoproterenol. These comparisons indicate little or no first-pass metabolism of isoproterenol following intrabronchial instillation of an aqueous solution of isoproterenol but suggest the possibility of a substantial first-pass effect when the drug was inhaled as an aerosol. The drug was also more rapidly absorbed following aerosol administration; it may be that this route of administration delivers the drug over a larger surface area in the airways than is achieved with intrabronchial administration. This may result in greater exposure of the drug to the epithelial cells of the bronchi and small airways, leading to more rapid absorption and avoiding local saturation of metabolizing capacity during absorption. This would hinder extrapolation of data following intrabronchial administration to situations involving aerosol inhalation of a drug and suggests that intrabronchial or endotracheal administration may be an inappropriate technique for studying drugs which are intended for aerosol administration.

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Isolated Perfused Rabbit Lung as a Model for Intravascular and Intrabronchial Administration of Bronchodilator Drugs II: Isoproterenol Prodrugs

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Abstract □ The pulmonary disposition of two diester prodrugs of isoproterenol (di-*p*-toluoylisoproterenol and dipivaloylisoproterenol) was studied in the isolated perfused rabbit lung preparation. High-pressure liquid chromatographic methods were developed to measure diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol from a single 1-ml perfusate sample. The prodrugs were administered directly into the circulating perfusion medium and by endotracheal instillation. Perfusate concentrations of diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol were measured for 180 min. The diesters were rapidly eliminated from the perfusate with a subsequent increase in monoester concentrations. Isoproterenol levels were observed within minutes of prodrug administration, peaked at 60–80 min, and declined slowly thereafter. The prodrugs were rapidly absorbed following endotracheal administration with 30–50% of the diester being metabolized during the first pass through the lung.

Keyphrases □ Isoproterenol prodrugs—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, high-pressure liquid chromatography □ Prodrugs—isoproterenol, isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, perfusion, high-pressure liquid chromatography □ Perfusion—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, high-pressure liquid chromatography □ High-pressure liquid chromatography—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, prodrugs, perfusion, isoproterenol

Isoproterenol is a β-adrenergic agonist which is often employed in the treatment of bronchial asthma. The drug has limited oral activity because of the extensive first-pass metabolism which occurs during absorption of the drug from the GI tract (1, 2). Aerosol inhalation is the most commonly used route of administration and offers the advantages of a rapid onset of activity and delivery of the drug directly to the airways (3). The duration of action following inhalation is short, however, and frequent dosing is often required to maintain the desired effect. This can result in side effects such as cardiac stimulation, which can hinder effective therapy with the drug. Increasing the duration of action of isoproterenol would offer a significant improvement in the clinical use of the drug for the treatment of asthma.

Prodrugs have been used in the past to improve the delivery of pharmacological agents, and this approach seems feasible with isoproterenol. An inactive derivative of isoproterenol, which itself possesses more favorable physicochemical and pharmacokinetic properties than the

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